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Hydrocortisone Attenuates Cyclosporin A-Induced Nephrotoxicity in Rats

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ABSTRACT

Cyclosporin A (CsA) is the prototype of immunosuppressant drugs that have revolutionized the management of all transplantation and autoimmune diseases. Side effects of CsA mainly affecting the kidney but also observed in liver and heart, limit the therapeutic use of this drug after organ transplantation. The renal toxicity of CsA is attributed to reduced renal blood flow which leads to hypoxia-reoxygenation injury accompanied by excessive generation of oxygen-derived free radicals. In several therapeutic protocols, CsA is used in association with corticosteroids to obtain better therapeutic results. Recently, our studies showed that hydrocortisone (HY) has a protective effect on CsA-induced cardiotoxicity. In fact our previous results demonstrated that in rat cardiomyocytes, CsA toxicity is due to a calcium overload, which in turn induce lipid peroxidation and determines oxidative stress-induced cell injury. Treatment with HY effectively inhibits CsA-induced toxicity, decreasing lipid peroxidation as well as calcium intracellular concentration. In this study we evaluated in vivo the effects of CsA, used alone or in association with HY, on some parameters of renal dysfunction (blood urea nitrogen; BUN, creatinine, and cholesterol), malondialdheyde (MDA) levels, antioxidant enzyme catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and apoptosis. CsA administration for 24 days resulted in a marked renal oxidative stress, which significantly deranged the renal functions. Treatment with CsA in association with HY significantly improved the renal dysfunction and renal oxidative status. This study clearly suggests the role of oxidative stress in the pathogenesis of CsA-induced nephrotoxicity. J. Cell. Biochem. 103: 997–1004, 2012.

KEY WORDS: CYCLOSPORIN A; SUPEROXIDE DISMUTASE; NEPHROTOXICITY; CATALASE; LIPID PEROXIDATION

Cyclosporin A (CsA) is a powerful immunosuppressive drug that is widely used to prevent organ rejection and to treat certain autoimmune disease [Hagar et al., 2006]. However, CsA treatment is often limited by severe adverse effects such as hypertension and nephrotoxicity [Banijamali et al., 1993; Taler et al., 1999]. Adverse renal effects of CsA can be divided into two categories: functional and histological abnormalities. CsA-induced renal dysfunction is related to reduced glomerular filtration rate and renal blood flow. This is generally thought to be secondary to vasoconstriction of the glomerular afferent arterioles, which causes a decrease in glomerular pressure [Andoh and Bennett, 1998]. Renal dysfunction, in turn, causes an increase in serum creatinine

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concentration and a decrease in creatinine clearance. These functional changes are dose-dependent and are usually reversible after short-term CsA treatment [Banijamali et al., 1993]. CsA-induced histological renal damage is mainly characterized by tubulointerstitial fibrosis of striped pattern and arteriolopathy of the afferent arterioles [Antonovych et al., 1988]. Interstitial fibrosis consists of streaks of fibrosis. Arteriolopathy is characterized by luminal narrowing and arteriolar wall thickening. These changes are composed of subendothelial edema, swelling of hypertrophied endothelial cells protruding into the lumen and eosinophilic granular transformation of vascular smooth muscle cells [Antonovych et al., 1988]. Moreover, the use of CsA is also reported to be related to increased vasoconstriction and vascular endothelial dysfunction [Stephan et al., 1995]. The exact mechanism of CsA-induced hypertension and nephrotoxicity remain obscure. Experimental studies revealed that several mechanisms may be involved. These include activation of the renin–angiotensin system (RAS) [Edwards et al., 1994] and increased synthesis of endothelins [Fogo et al., 1992]. Several studies suggest that a defect in intracellular calcium handling [Lo Russo et al., 1996], magnesium deficiency [Mervaala et al., 1997], oxidative stress [De Nicola et al., 1993], and nitric oxide system [Navarro-Antolin et al., 1998] are involved. Although there are several possible mechanism of CsA-induced nephrotoxicity, it has been proposed that CsA-related toxicity may in part be due to excessive generation of reactive oxygen species (ROS) [Wang and Salahudden, 1994; Wolf et al., 1997]. Furthermore, cell and tissue lipid peroxidation induced by cyclosporin as well as the release of ROS has recently been pointed out to be the factors responsible for the toxic phenomena related to the administration of cyclosporin [Longoni et al., 1999]. Several lines of evidence suggest that CsA-induced renal and cardiovascular toxicity may be due to oxidative stress. In fact, CsA increases hypoxia and free radical production in the rat kidney [Zhong et al., 1998]. Antioxidants, such as α-tocopherol, ascorbate, lazaroids, and superoxide dismutase (SOD)/catalase (CAT), have been shown to decrease CsA-induced renal toxicity [Wang and Salahudden, 1994]. In several therapeutic protocols CsA is used in association with corticosteroids to obtain better therapeutic results. In fact, many evidence suggest that the hydrocortisone (HY), a steroidal anti-inflammatory drug is able to reduce lipid peroxidation induced in rat by ligated loop of the distal ileum [Otamiri, 1989] or in the rat hyppocampus under stress conditions [Tolstuckima et al., 1999]. HY also has a strong immunosuppressive activity. It evokes immunosuppression by inducing apoptosis of T lymphocytes by activating supposed lysis genes or by repressing the gene expression required for proliferation and growth [Cohen, 1991]. Our previous research demonstrated that, in rat cardiomyocytes, HY effectively inhibits CsA-induced toxicity [Florio et al., 2003] and that the balance between lipid peroxidation products and nitric oxide production may play a key role to increase drug-induced cytotoxicity [Pacilio et al., 1998; Pagnini et al., 2000a, 2000b]. With this in mind, the aim of the present study was to evaluate in vivo in kidney tissue the effects of CsA used alone or in association with HY on some parameters of renal dysfunction, on oxidative stress and apoptosis.

MATERIALS AND METHODS

ANIMALS

Adult male Wistar rats weighing 180–200 g (Harlan-Nossan-Milan, Italy) were housed in a 12-h dark/light cycle animal facility with controlled temperature and humidity. Food and water were given ad libitum throughout the study. Every effort was made to minimize animal suffering and the studies were performed according to Italian Law on the protection of laboratory animals "Decreto legislativo n. 116 del 27/01/1992. Attuazione della direttiva (CEE) n. 609/86 in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici," all procedures were approved by the Italian Ministry of Health.

CHEMICALS

CsA (Sandimmun) was obtained from Novartis Farma S.p.A. (Origgio, Varese, Italy); HY and Malondiadheyde were purchased from Sigma (Milan, Italy). The drug solutions were freshly prepared and used on the same day.

EXPERIMENTAL PROTOCOL

The rats were randomized and divided into four groups of 10 animals each and were treated for 24 days as follows: the first group (control) received a daily equivalent volume of vehicle for CsA, castor oil, subcutaneously (s.c.). The second group received CsA (25 mg/kg/s.c./die/2 ml) dissolved in castor oil, the third group received intraperitoneally (i.p.) HY (5 mg/kg/i.p./die/2 ml); the fourth group received CsA (25 mg/kg/s.c./die/2 ml) associated to HY (5 mg/kg/i.p./die/2 ml). Diastolic blood pressure (DBP) and systolic blood pressure (SBP) was measured from the tail of the animals using a blood pressure recorder (BP Recorder mod. LE 5002 Ugo Basile, Italy) on days 0–6–12–18–24. The animals were adapted to the BP recording for at least 5 days before starting the experiments. Twenty-four hours after last treatment, the animals were sacrificed and blood was collected for various biochemical assay. A midline abdominal incision was performed and both the kidneys were isolated, the left kidney was immediately removed and stored at −70°C for the estimation of lipid peroxidation, glutathione peroxidase (GPx), CAT SOD activity, and apoptosis. The right kidney was fixed in 10% neutral-buffered formalin for histological studies. The blood was collected through abdominal aorta. The blood samples were processed to harvest the BUN, creatinine, and cholesterol and were assayed using standard diagnostic kits (Sigma).

RENAL TISSUE PREPARATION

Renal tissue was homogenized in a solution of 50 mM potassium phosphate, 0.1% Triton (pH 7.0). Crude homogenates were centrifuged at 15,300 rpm for 30 min at 4°C and used to assess antioxidant enzyme activities, tissue nitrite concentration, and apoptosis assay.

LIPID PEROXIDATION ASSAY

Lipid peroxidation as index of the oxidative stress was determined by assaying the malondialdheyde (MDA) production by means of the thiobarbituric acid (TBA) test [Esterbauer and Cheeseman, 1990]. Briefly: 1 ml of supernatant of renal tissue treated or untreated with

CsA, HY, and CsA + HY was mixed with 0.5 mL of cold 30% (w/v) trichloroacetic acid to precipitate proteins. The precipitate was pelletted by centrifugation and 1 ml of the supernatant was reacted with 1.3 ml of 0.5% (w/v) TBA at 85°C for 40 min. In TBA test reaction, one molecule of MDA reacts with two molecules of TBA with the production of a pink pigment having maximal absorbance at 532–535 nm. Therefore, after cooling, the fluorescence was read at an excitation of 536 nm and an emission of 557 nm wavelengths in a SPEX Fluoromax spectrophotofluorimeter. The concentration of MDA was calculated respect to a calibration curve (range: 0.5–2 pmoles/ml), and results were expressed as pmoles of MDA/mg proteins/24 h.

SOD ACTIVITY ASSAY

This assay for SOD activity involves inhibition of nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase used as a superoxide generator [Sun et al., 1988]. SOD is one of the most important antioxidative enzymes. It catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. SOD activity was determined by the sensitive SOD assay that utilizes a product a water-soluble formazan dye upon reduction with superoxide anion SOD Activity with absorbance at 450 nm (Assay Kit Fluka Products). Briefly, 1 ml of supernatant of renal tissue treated or not treated with CsA, HY and CSA + HY was mixed. At the end of incubation the supernatant were lysed and centrifuged at 14,000 × *g* for 5 min at 4°C and on cytosol was determined cytosolic and mitochondrial SOD activity. The starter of reaction by adding Xanthine Solution. The absorbance readings every minute for 10 min at room temperature. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. The SOD activity was expressed as % of inhibition respect to activity control. The % inhibition of the rate of increase in absorbance is calculated as follows: % Inhibition = (slope of activity control − slope of sample) × 100. The amount of SOD that inhibited NBT reductionly 50% was defined to be 1 unit of enzyme activity.

CAT ACTIVITY ASSAY

CAT activity. The colorimetric assay, described by Sinha [1972] has been applied to evaluate CAT activity. The method is based on the principle that dichromate in acetic acid is reduced to chromate acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically and absorbance was read at 520 nm. Results are expressed as units for milligrams of protein.

GPx ACTIVITY ASSAY

GPx activity. GPx activity was indirectly detected by a method previously described by Lawrence and Burk [1976]. GPx requires reduced glutathione, which is regenerated by glutathione reductase using oxidized glutathione, a process that consumes NADPH. The activity of GPx is defined as micromoles of NADPH oxidized per minute, taking into account that the millimolar absorption coefficient for NADPH at 340 nm is 6.22 L $mmol^{-1}$ cm^{-1}. Results are expressed as units for milligrams of protein.

APOPTOSIS ASSAY

To detect mono and oligo-nuclesomal DNA fragmentation, the Cell Death Detection ELISA Plus (Boehringer Mannheim, Indianapolis, IN) was used. The assay is based on a quantitative sandwich-immunoassay principle using mouse monoclonal antibodies direct against DNA and histones, respectively. Cytosol of kidney, containing the fragmented DNA, were obtained by centrifugation added to each well of 96-well plates coated with antihistone antibody. After 90 min of incubation at room temperature, the wells were washed three times with PBS. Peroxidase conjugated anti-DNA antibody was added to the wells, and incubated for 90 min at room temperature. After the wells were washed three times, 100 ml of colorimetric substrate was added and serial readings of optical density at 405 and 490 nm was taken at 5 min intervals.

TOTAL PROTEIN ASSAY

Total proteins were determined by the method of Lowry et al. [1951].

STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation of 10 independent determinations performed in duplicates. An error probability with $P < 0.05$ ($P < 0.05$ (including $P < 0.01$ and 0.001) was selected as significant. All experiments were performed in duplicates and the mean was used for analysis.

RESULTS

Figure 1 shows the values of blood pressure. Both CsA that HY-induced hypertension in a time-dependent manner after 12 days of treatment. After 24 days, in rats treated with CsA the SBP shifted from 109.2 ± 3.9 to 132.4 ± 5.8 mmHg and DBP shifted from 95.1 ± 3.2 to 117.3 ± 5.3 mmHg while in rats treated with HY the SBP shifted from 107.3 ± 3.1 to 123.2 ± 5.6 mmHg and DBP shifted from 92.1 ± 3.6 to 106.5 ± 5.8 mmHg. The combination (HY + CsA) induced an increase in SBP and DBP from 123.2 ± 5.6 mmHg (HY alone) or 132.4 ± 5.8 mmHg (CsA alone) to 141.5 ± 6.1 mmHg, while the DBP increased from 106.5 ± 4.2 mmHg (HY alone) or 117.3 ± 5.7 mmHg (CsA alone) to 129.4 ± 5.3 mmHg.

Table I shows the results of BUN levels of rats treated or untreated for 0, 6, 12, 18, and 24 days with CsA or with HY alone or in combination. The treatment with various drugs up to 18 days did not produce any significant change in BUN levels while treatment with CsA for 24 days was able to significantly increase the BUN levels whose values shifted from 26.8 ± 2.9 to 45.6 ± 2.6 mg/dl. The treatment with HY after 24 days did not induce any significant change in BUN levels and values shifted from 29.7 ± 4.8 to 37.8 ± 5.1 mg/dl. The HY used in association with CsA was able to significantly reduce the CsA-induced BUN increase shifting the values from 45.6 ± 2.6 mg/dl (CsA alone) to 37.8 ± 3.4 mg/dl (CsA + HY).

Table II shows the results on cholesterol levels of rats treated or untreated for 0, 6, 12, 18, and 24 days with CsA or with HY alone or in association. The treatment with various drugs up to 18 days did not produce any significant change in cholesterol levels while

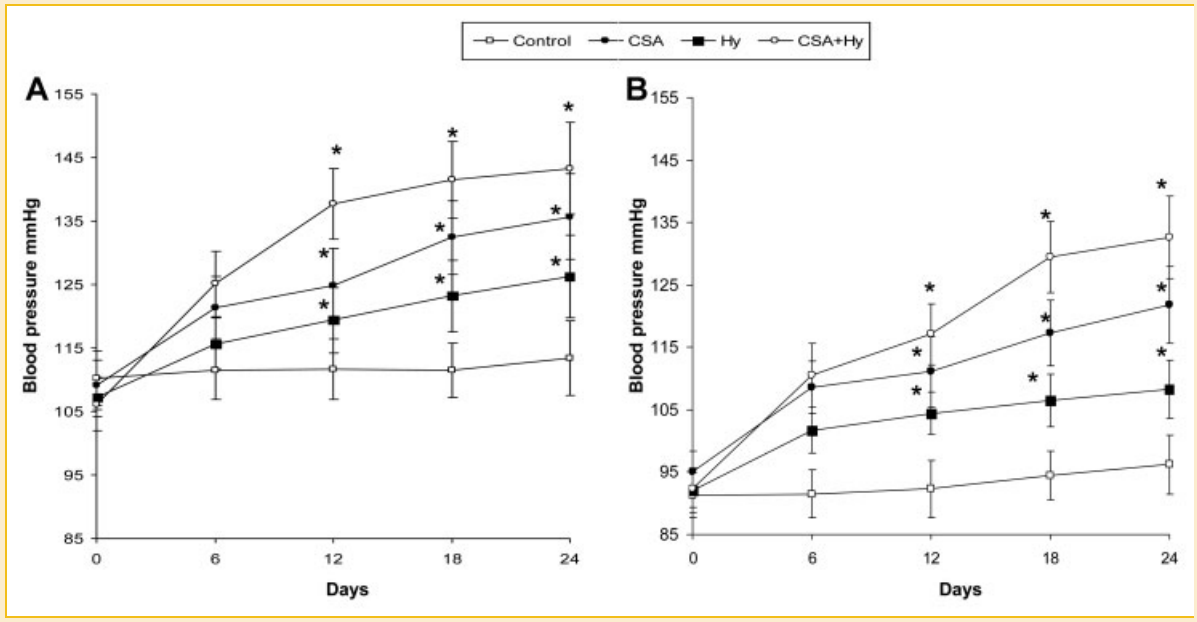

Fig. 1. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) used alone or in association on blood pressure values systolic (A) and diastolic (B) at 0–6–12–18–24 days of treatment. Data represents mean ± SD of 10 animals. $^{*}P < 0.05$ versus control.

treatment with CsA for 24 days was able to significantly increase plasma cholesterol levels whose values shifted from 54.3 ± 723 to 75.6 ± 9.32 mg/dl. Treatment with HY after 24 days of treatment did not induce any significant change with values that varied from 51.1 ± 5.3 to 53.1 ± 4.4 mg/dl. The HY used in combination with CsA was able to significantly reduce the CsA-induced increase shifting the cholesterol values from 75.6 ± 9.3 mg/dl (CsA alone) to 59.8 ± 5.3 mg/dl (CsA + HY).

Table III shows the results obtained on serum creatinine levels in rats treated or untreated for 0, 6, 12, 18, and 24 days with CsA or with HY alone or in combination. Even on this parameter, the treatment with various drugs up to 18 days did not produce any significant change while treatment with CsA for 24 days was able to significantly increase the creatinine levels whose values shifted from 0.35 ± 0.02 to 0.46 ± 0.01 mg/dl of creatinine. Treatment with HY after 24 days of treatment did not induce any significant change in serum creatinine (from 0.36 ± 0.04 to 0.38 ± 0.02 mg/dl of creatinine). HY used in association with CsA was able to significantly reduce the increase in creatinine induced by CsA whose values shifted from 0.46 ± 0.01 mg/dl (CsA only) to 0.38 ± 0.04 mg/dl creatinine (CsA + HY).

CATALASE

Figure 2 shows the results on the CAT activity in kidney of rats treated or untreated for 12 and 24 days with CsA or with HY alone or in association. After 12 days of treatment with various drugs we did not found any significant variation in the CAT levels, while treatment with CsA for 24 days was able to significantly increase the CAT activity in kidney from 0.42 ± 0.032 to 0.8 ± 0.054. The treatment with HY alone had not significant effect on CAT activity, with values shifted from 0.42 ± 0.042 to 0.51 ± 0.053 while was able to inhibit the effect of CsA by reducing significantly the values by 0.8 ± 0.054 (CsA alone) to 0.71 ± 0.046 (CsA + HY).

SUPEROXIDE DISMUTASE

Figure 3 shows the results obtained on the SOD activity in rats treated or untreated for 12 and 24 days with CsA or with HY alone or in combination. After 12 days of treatment with various drugs no

TABLE I. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) Used Alone or in Association on Plasma Urea at 0–6–12–18–24 Days of Treatment

	Days				
Treatment	0	6	12	18	24
Control	26.8 ± 4.5	24.8 ± 3.5	28.2 ± 2.9	29.6 ± 3.3	30.3 ± 2.7
CSA	26.8 ± 2.9	36.4 ± 4.6	35.9 ± 3.9	39.5 ± 4.8	45.6 ± 2.2*
HY	29.7 ± 4.8	35.1 ± 3.9	32.6 ± 3.1	33.7 ± 4.3	37.8 ± 5.1
CSA + HY	26.1 ± 2.9	31.9 ± 4.8	31.5 ± 4.4	36.1 ± 2.9	37.8 ± 3.0**

Data are expressed as urea mg/dl and mean ± SD of 10 animals. $^{*}P < 0.05$ versus control. $^{**}P < 0.05$ versus CSA alone.

TABLE II. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) Used Alone or in Association on Blood Cholesterol at 0–6–12–18–24 Days of Treatment

	Days				
Treatment	0	6	12	18	24
Control	53.9 ± 6.1	52.8 ± 5.8	54.6 ± 4.9	54.2 ± 6.0	54.3 ± 5.8
CSA	54.3 ± 7.2	56.3 ± 5.5	50.6 ± 5.3	56.4 ± 6.2	75.6 ± 9.3*
HY	51.1 ± 5.3	52.9 ± 5.4	56.8 ± 6.0	55.1 ± 5.9	53.1 ± 4.4
CSA + HY	52.8 ± 4.9	53.1 ± 5.7	51.6 ± 4.6	53.8 ± 5.7	59.8 ± 5.3**

Data are expressed as cholesterol mg/dl and mean ± SD of 10 animals. $^{*}P < 0.05$ versus control. $^{**}P < 0.05$ versus CSA alone.

TABLE III. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) Used Alone or in Association on Serum Creatinine Obtained to 0–6–12–18–24 Days of Treatment

	Days				
Treatment	0	6	12	18	24
Control	0.34 ± 0.03	0.36 ± 0.02	0.35 ± 0.02	0.37 ± 0.03	0.36 ± 0.03
CSA	0.35 ± 0.02	0.34 ± 0.02	0.37 ± 0.02	0.41 ± 0.02	$0.46 \pm 0.01^{*}$
HY	0.36 ± 0.04	0.35 ± 0.12	0.36 ± 0.12	0.37 ± 0.02	0.38 ± 0.02
CSA + HY	0.34 ± 0.02	0.33 ± 0.02	0.38 ± 0.03	0.38 ± 0.05	$0.38 \pm 0.04^{**}$

Data are expressed as creatinine mg/dl and mean ± SD of 10 animals. $^{*}P < 0.05$ versus control. $^{**}P < 0.05$ versus CSA alone.

significant variation in the SOD levels was observed, while treatment with CsA for 24 days was able to significantly reduce the SOD activity in kidney. In fact, the % of inhibition shifted from 63.1 ± 8.1 to 32.5 ± 4.3. Treatment with HY alone did not change significant effect, with values varying from 63.1 ± 8.1 to 62.3 ± 6.9. The HY used in combination with CsA was able to increase the SOD activity with values shifted from 32.5 ± 4.3 (CsA alone) to 41.2 ± 5.3 (CsA + HY).

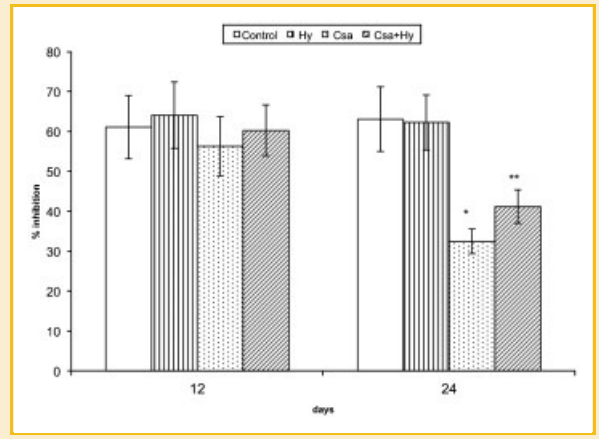

Fig. 3. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) used alone or in association on SOD levels in rat kidney after 12 and 24 days of treatment. Data represents mean ± SD of 10 animals. $^{*}P < 0.05$ versus control. $^{**}P < 0.05$ versus CSA alone.

GLUTATHIONE PEROXIDASE

Figure 4 shows the results obtained on the GPx activity in rats treated or untreated for 12 and 24 days with CsA or with HY alone or in combination. After 12 days of treatment with various drugs no significant change was found in GPx levels while treatment with CsA for 24 days was able to significantly reduce the activity of GPx in the kidney from 0.016 ± 0.0031 to 0.008 ± 0.0011.The treatment with HY alone did not significant effect (from 0.016 ± 0.0031 to 0.015 ± 0.0012), while the HY used in combination with CsA was able to increase the CsA effects decreasing GPx values from 0.008 ± 0.0011 U/mg (CsA alone) to 0.011 ± 0.009 U/mg (CsA + HY) of proteins.

MALONDIALDEHYDE

Figure 5 shows the results obtained in rats treated or untreated for 12 and 24 days with CsA or with HY used alone or in combination on MDA levels. Administration of CsA produced an increase in the levels of MDA after 12 days which became significant after 24 days of treatment increasing from 149.7 ± 27.5 to 283.4 ± 33.9 pmoles/mg of proteins. HY used alone did not produce significant changes (from 149.7 ± 27.5 to 151.7 ± 21.9 pmoles/mg of proteins) but when HY was used in combination with CsA was able to reduce the CsA activity decreasing the MDA levels from 283.4 ± 33.9 to 179.3 ± 19.2 pmoles/mg of proteins.

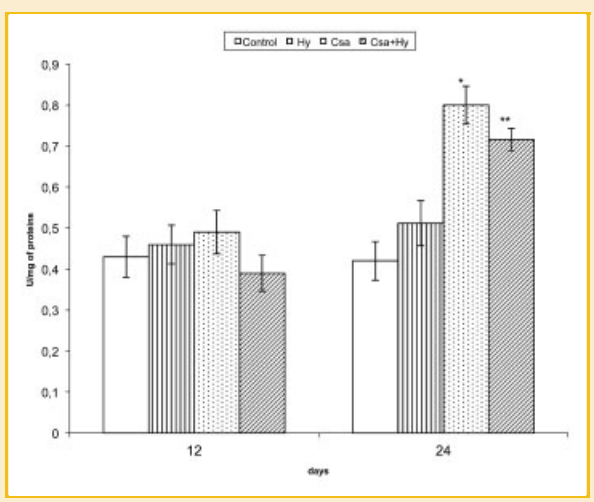

Fig. 2. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) used alone or in association on CAT levels in rat kidney after 12 and 24 days of treatment. Data represents mean ± SD of 10 animals. $^{*}P < 0.05$ versus control. $^{**}P < 0.05$ versus CSA alone.

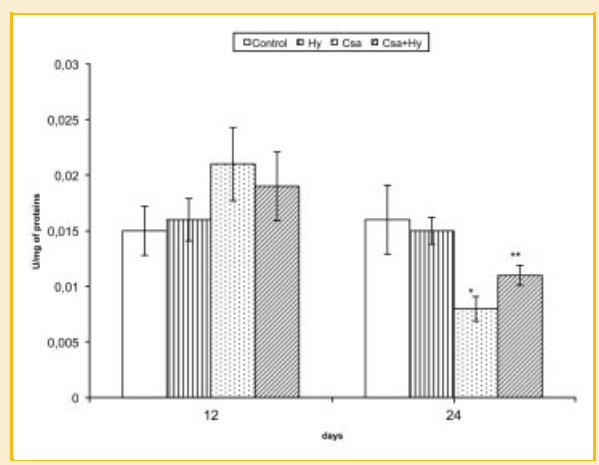

Fig. 4. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) used alone or in association on GPx levels in rat kidney after 12 and 24 days of treatment. Data represents mean ± SD of 10 animals. $^{*}P < 0.05$ versus control. $^{**}P < 0.05$ versus CSA alone.

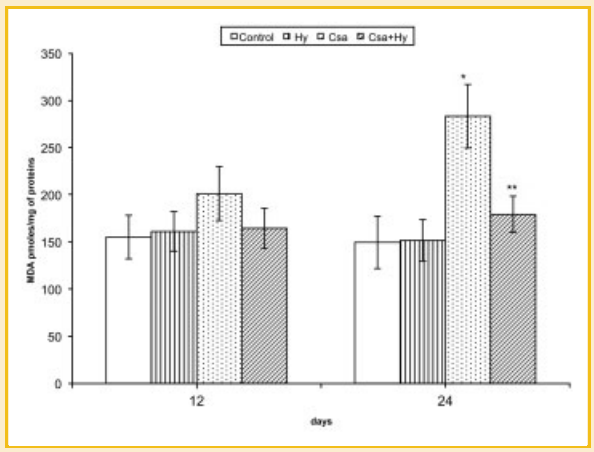

Fig. 5. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) used alone or in association on lipid peroxidation (MDA) in rat kidney after 12 and 24 days of treatment. Data represents mean ± SD of 10 animals. $^{*}P < 0.05$ versus control. $^{**}P < 0.05$ versus CSA alone.

APOPTOSIS

The apoptotic activity of different treatment protocols was evaluated by assaying the levels of oligonucleosomes, in the cytosol of kidney tissue. Figure 6 shows the absorbance values obtained in the cytosol of kidneys of rats treated or untreated for 12 or 24 days with CsA or HY alone or in combination. The treatment of various drugs for 12 days did not determined any significant change. Treatment with CsA for 24 days resulted in a significant increase in the apoptotic activity shifting absorbance values from 0.28 ± 0.03 to 0.65 ± 0.073. HY treatment did not induce any significant change in absorbance values (from 0.28 ± 0.03 to 0.34 ± 0.042) but used in combination with CsA was able to significantly reduce the CsA induced increase in absorbance shifting from 0.65 ± 0.073 (CsA alone) to 0.43 ± 0.052 (CsA + HY).

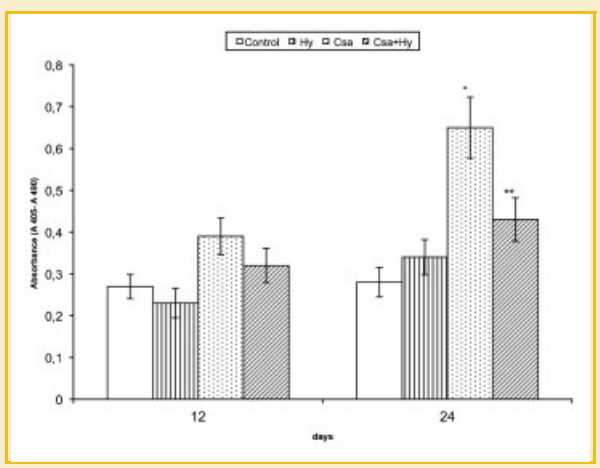

Fig. 6. Effects of CsA (25 mg/kg/s.c./die) or HY (5 mg/kg/i.p./die) used alone or in association on intracytosolic levels of nucleosomes in rat kidney after 12 and 24 days of treatment. Data represents mean of absorbance ± SD of 10 animals. $^{*}P < 0.05$ versus control. $^{**}P < 0.05$ versus CSA alone.

DISCUSSION

Therapy with CsA can lead to functional and structural changes in the kidney of transplanted patients and in experimental animals leading to renal dysfunction [Jackson et al., 1987] inducing a dose-related in renal function. Histopathological changes including interstitial fibrosis and proximal and/or distal tubular damage have been reported following treatment with CsA for extrarenal diseases [Mason, 1990]. Our studies showed that chronic administration of CsA caused a increase of blood pressure and a marked impairment of renal function increasing significantly the creatinine, cholesterol, BUN levels, and inducing morphostructural changes of the tubular vacuoles and necrosis, interstitial fibrosis, and parietal cell hyperplasia while HY did not led to enhancements (unpublished data). HY treatment increased blood pressure, but significantly reduced CsA-induced effects on BUN, creatinine, and cholesterol. A relationship between nephrotoxicity and oxidative stress has been confirmed in many experiments. In fact several antioxidants have been used to attenuate CsA-induced nephrotoxicity [Naidu et al., 1999; Tariq et al., 1999; Padi and Chopra, 2002]. Melatonin, a potent hydroxyl radical scavenger, protected the CsA induced renal tubular damage [Naidu et al., 1999]. Carvedilol, a beta-blocker with potent free radical scavenger activity, reduced the MDA levels and improved the renal dysfunction and morphological changes induced by CsA [Padi and Chopra, 2002]. Co-administration of catechin with CsA significantly reduced the lipid peroxidation and restored the decreased glutathione levels induced by CsA [Anjaneyulu et al., 2003]. The activity of glucocorticoids on oxidative stress has not been well demonstrated. Infact Rajashree and Puvanakrishnan [1998] have reported that antioxidant enzymes such as CAT, SOD, GPx, and glutathione S-transferase exhibited a significant decrease in heart during dexametasone administration, whereas, in kidney, they exhibited a significant increase during treatment and after withdrawal of dexametasone. Recently, it has been shown that chronic isolation caused decreased in serum corticosterone and a disproportion between CuZnSOD, peroxidases (CAT and GPx) and GLR, thus promoting H_2O_2 accumulation and prooxidative state in liver [Djordjevic et al., 2010]. In our study, we have shown that chronic treatment with CsA significantly reduced SOD and Gpx activities in kidney, which accounts for the increased susceptibility to oxidative stress of the cellular structures. Decreased SOD and Gpx activities are probably due to the reduced synthesis or to an increase of the turn-over of such enzymes [Deman et al., 2001; Yilmaz et al., 2006]. In our experiments, though it has been demonstrated an increase of CAT activity in the kidneys treated with CsA, the levels of MDA were significantly increased and HY was able to reduce oxidative stress by reducing the levels of MDA and reverting the CsA effect on SOD and GPx activity. Tubulointerstitial injury is the prominent feature of chronic CsA nephropathy, and the major form of cell death is apoptosis [Yang et al., 2002]. Apoptosis is an active mechanism of cell clearance and plays a key role in the regulation of cell number during development, in tissue homeostasis, and following insults. In the kidney, apoptosis may be beneficial [Ortiz et al., 2000] but is deleterious if enough resident cells are lost [Li et al., 2002]. CsA has been shown to induce apoptotic cell death not only in T-lymphocytes, thus interfering with T-cell function [Ying

et al., 2003], but also in some renal cells, resulting in the deterioration of kidney structure. The literature data concerning the effect of glucocorticoids on apoptosis are discordant and depend on the tissue and the disease. The HY has a strong immunosuppressive activity, in fact evokes immunosuppression by inducing the apoptosis of T lymphocytes by activating supposed lysis genes or by repressing the gene expression required for proliferation and growth [Mazzali et al., 2001; Yang et al., 2003]. Several reports show that the activity of glucocorticoids is different depending on the cell line, infact cause cell cycle arrest and apoptosis in lymphoid cells [Wasim et al., 2010], in carcinoma cells [Herr et al., 2003], instead suppresses CsA-induced apoptosis but not cell cycle arrest in MDCK cells [Jeon et al., 2005]. Our results showed that CsA administered in rats for 24 days was able to induce apoptosis in renal cell and that HY administered in combination reduced CsA induced apoptosis. Our results suggest that the effect of HY on CsA-induced apoptosis cold be due to its anti-oxidant effect. In conclusion, our data demonstrate that, in rat kidney, CsA toxicity is due to an oxidative stress overload which in turn induce lipid peroxidation and determines oxidative stress-induced cell injury. Treatment with HY effectively inhibits CsA-induced toxicity, decreasing lipid peroxidation as well as apoptosis. Our findings seem to suggest that glucocorticoids may be effective in reducing CsA-induced nephrotoxicity at concentrations consistent with therapeutic doses.

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